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The Pharmacology of Semisynthetic Antibiotics

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ABSTRACT

The chemical structures and reactions of penicillins and cephalosporins are reviewed in relation to their effects upon pharmacodynamic properties. The reactive beta-lactam ring is common to all penicillins and cephalosporin C analogues. This ring opens during acylation of the bacterial wall-building enzymes, but previous opening of the ring by acid or beta-lactamases destroys antibiotic activity.

Semisynthetic substitutions may protect the ring by steric hindrance; this may actually inactivate certain penicillinases, so that resistant penicillins may potentiate penicillin G in some circumstances. However, the protective substitutions reduce the intrinsic activity of the synthetic penicillins themselves. Other properties which are affected include absorption, protein-binding, excretion, and possible allergenicity of the drugs. Effects on antibacterial spectrum may possibly be secondary to alteration of lipid solubility.

SOMMAIRE

L'article passe en revue les structures et réactions chimiques des pénicillines et des céphalosporines en rapport avec leurs propriétés pharmacodynamiques. Le noyeau bêta-lactame actif est commun à toutes les pénicillines et aux analogues de la céphalosporine C. Ce noyeau s'ouvre au cours de l'acylation des enzymes qui président à l'édification des membranes cellulaires des bactéries, mais son ouverture prématurée sous l'influence d'un acide ou de bêta-lactamases détruit l'activité antibiotique.

Les substitutions effectuées sur les antibiotiques semisynthétiques peuvent protéger le noyeau par empêchement stérique; en fait, ceci peut inactiver certaines pénicillinases, de sorte que certaines pénicillines résistantes parviennent à potentialiser la pénicilline G en certaines circonstances. Cependant, les remplacements protecteurs réduisent l'activité intrinsèque des pénicillines synthétiques elles-mêmes. Parmi les autres propriétées qui sont touchées, figurent l'absorption, la liaison aux protéines, l'excrétion et la possibilité que le médicament devienne allergène. Il est possible que les effets de ces substitutions sur le spectre antibactérien soient secondaires à une modification de la solubilité des lipides.

IN A sense, the subject indicated in the foregoing title is an impossible one to cover because it is almost impossible to define. Semisynthetic antibiotics are, in effect, almost as old as antibiotics themselves, because minor modifications of structure were rapidly introduced for the purpose of affecting solubility and thereby altering the routes and schedules of administration. An early example was the formation of various salts or esters of penicillin, such as those with aluminum, procaine or benzathine. All of these, being poorly soluble, can be used as depot forms for intramuscular injection, to give slow and even absorption and therefore prolonged action. The addition of a pyrrolidinomethyl group to the tetracyline structure, giving the product known as rolitetracycline, resulted in

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a sufficiently high solubility to permit intravenous administration of an adequate dose. A more recent modification, lymecycline, achieves essentially the same goal by combining the tetracycline with a derivative of the aminoacid lysine.[1] Strictly speaking, all such modifications result in semisynthetic antibiotics.

Usually, however, the term is reserved for those modifications of the basic structure of the antibiotics which affect more fundamental chemical properties, such as those which determine the stability in acids and alkalis, the stability against enzymatic degradation, and the spectrum of antibacterial activity. Improvements based on such modifications have so far been achieved mainly among the penicillins and cephalosporins; modifications of other antibiotics are not yet so numerous or sophisticated as these. Therefore, the material to be covered in this review will deal mainly with penicillins and cephalosporins.

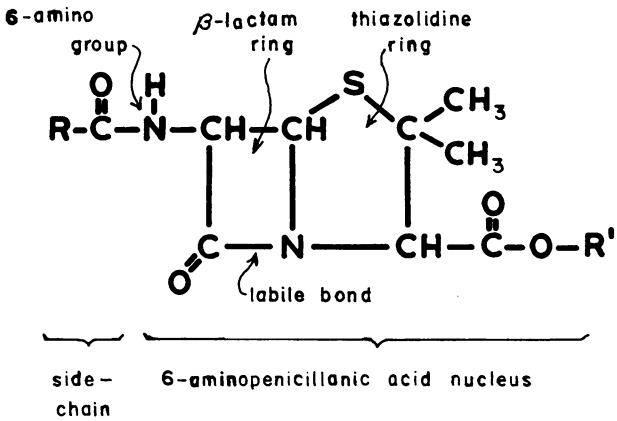

Fig. 1.—Basic chemical structure of penicillins.

Fig. 1 shows the basic structure of penicillins, 6-aminopenicillanic acid. The letter R′ indicates the site at which salt or ester formation takes place. If, instead of being a hydrogen ion as in penicillanic acid, R′ is a Na^+ or K^+ ion, the products are soluble penicillin salts. If R′ is benzathine, procaine, or aluminum, as mentioned earlier, the products are relatively insoluble. But the important parts of the molecule with respect to semisynthetic modifications are the β-lactam ring and the group designated by the letter R. The four-membered β-lactam ring is a relatively unstable structure because it is under a great deal of internal strain, and is therefore highly reactive. This is fundamental to the action of the penicillins, as will be explained below. The stability of this structure, however, is greatly influenced by the nature of the R side-chain attached to the 6-amino group. It is the deliberate modification of the R side-chain which gives rise to the semisynthetic penicillins.

The cephalosporins contain a very similar basic structure (Fig. 2), except that it has a six-membered dihydrothiazine ring in place of the five-membered thiazolidine ring of the penicillins.

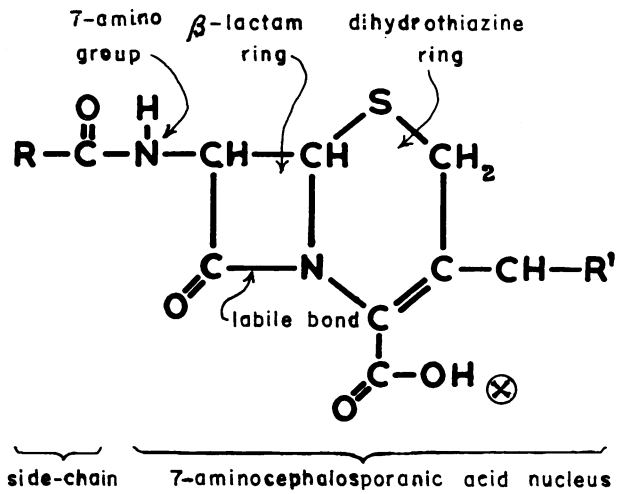

Fig. 2.—Basic chemical structure of cephalosporin C and related compounds. ⊗ indicates site of salt formation.

Simple salt formation occurs by replacement of the H atom marked ⊗. Both R and R′ are important side-chains, which are modified in the production of semisynthetic cephalosporin derivatives.

The essential portion of both molecules, from the point of view of their antibacterial action, is the β-lactam ring. It is this ring which is involved when the antibiotic reacts with the susceptible site in the bacteria; it is also this ring which is broken when the penicillin is degraded by acid and by the most common types of penicillinase. The action of penicillins and cephalosporins involves the breaking of the labile C-N linkage in the β-lactam ring, and the attachment of the C to some receptor site on the bacteria by a strong covalent bond. This process is called acylation, and the chemical groups in the bacteria which become acylated include some which are essential for the formation of bacterial cell walls.

All living cells, plant and animal alike, are surrounded by thin cytoplasmic membranes which are highly organized structures with specialized metabolic functions, including enzymatic activities involved in the transport of materials back and forth across the cell membrane. In addition to this thin cytoplasmic membrane, bacteria and plant cells have a heavy rigid outer wall which gives them mechanical strength to resist physical and osmotic shock. The latter point is particularly important because bacteria have a much higher internal osmotic pressure than do animal cells; without the protection of their rigid outer wall, bacteria in isotonic body fluids absorb water, swell up, and burst. The material of which the outer wall is built is synthesized inside the bacteria and then transferred to the outside and incorporated into the new wall of the growing bacteria, by enzymes located in the bacterial cytoplasmic membrane. Penicillins and cephalosporins acylate some group or groups in these transferring enzymes, thus blocking further synthesis of the outer wall. The bacteria continue to grow and divide, but with in-

complete outer walls they are subject to osmotic destruction, which is the basis of the bactericidal action of penicillins and cephalosporins. The formation of covalent bonds is readily demonstrable by labelling the penicillin with radioactive sulfur; when the penicillin acts on the bacteria the S^{35} is fixed to their cell membranes and cannot be washed off. One can prevent its attachment by first treating the bacteria with other acylating reagents, which form the same type of bonds and block the sites of uptake on the cell membrane so that the penicillin cannot react. The same holds true for cephalosporins.

The other point is that this same break in the β-lactam ring occurs when penicillinases act on susceptible penicillins. Hydrolysis of the same C-N linkage, whether by acid or by penicillinase, gives rise to a substance known as penicilloic acid (Fig.

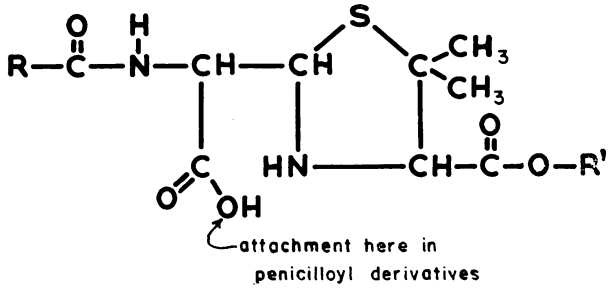

Fig. 3.—Chemical structure of penicilloic acid and penicilloyl derivatives. Penicilloyl-polylysine is not made by direct reaction with penicilloic acid, but by condensation with penicillanic acid, which rearranges to give the penicilloyl derivative.

3). This substance can no longer form the same type of bond with the receptor site; it can now form only salts or esters and is therefore inactive as an antibacterial agent. The cephalosporins, which have the same type of structure, are not sensitive to the same enzymes which split penicillins, but similar enzymes known as cephalosporinases do the same type of thing to the β-lactam ring of cephalosporins.[2]

The side chains (R) have a marked influence upon the stability of the β-lactam ring, and can therefore influence the properties of the penicillins in several ways. The first semisynthetic modification which proved clinically useful was to replace the benzyl R group of penicillin G with other side-groups (phenoxyethyl, phenoxymethyl, etc.) which tend to change the electron distribution in the lactam ring in such a way as to make it less reactive with H^+ and therefore more stable in the stomach.

The more recent modifications, however, have involved the use of R groups which are sufficiently bulky and so arranged as to shield the labile C-N bond, a process known as *steric hindrance*. This is difficult to illustrate in a chemical formula drawn on a flat surface. However, an attempt to represent this three-dimensionally (Fig. 4) will perhaps be a little more successful. If R = dimethoxyphenyl

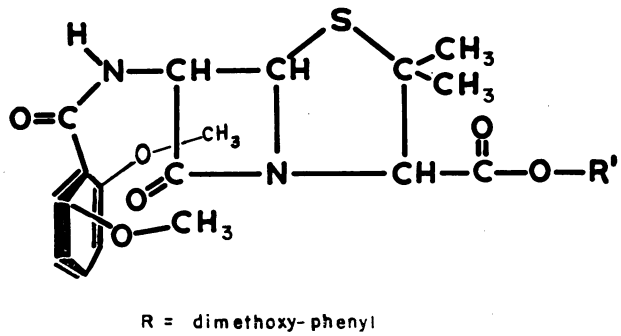

Fig. 4.—Chemical structure of methicillin (dimethoxyphenyl penicillin), showing steric hindrance of reactive bond of β-lactam ring by the two methoxy (-O-CH_3) groups.

(methicillin), the position of the methoxy groups can be described as enveloping the reactive area of the β-lactam ring, thus interfering with its close access to the catalytic site of the penicillinase. With ordinary penicillin G, the combination with the penicillinase occurs in a specifically oriented way: the two molecules must be aligned in the right position so that they can approach each other very closely and interact. When they do so, the C-N bond of the penicillin is split, and inactive penicilloic acid separates from the enzyme, which is then free to take up another molecule of penicillin. Methicillin and oxacillin, however (and other similar semisynthetics), combine with the enzyme but the steric hindrance prevents the molecules from aligning in the correct way to split the C-N bond. Instead, the penicillin remains very firmly bound to the enzyme, and it is the enzyme itself which somehow becomes pushed out of shape by the side-groups of the penicillin, losing its enzymatic activity.[3] It has been shown that penicillinases produced by a number of different bacteria are inactivated in this way by methicillin, oxacillin and cloxacillin. As a result, these new penicillins may perhaps potentiate the action of penicillin G against such bacteria.[4]

This last feature may prove to be of clinical value, because penicillin G remains the most active of all penicillins against susceptible bacteria. Probably the same steric hindrance which protects the β-lactam ring against penicillinase also impairs its ability to react with the target sites in the bacterial cell membrane. Therefore the penicillinase-resistant penicillins have a considerably lower intrinsic activity than penicillin G. It is conceivable that their lower activity may also be due in part to a slower diffusion through the outer cell wall to the site of attachment on the cytoplasmic membrane, because one can enhance the effectiveness of these penicillins by increasing the concentration.

Other properties of the penicillins which are modified by the semisynthetic alterations of side-chain have to do with the interaction between the drug and the patient, rather than the drug and the bacteria. For example, absorption across the gastrointestinal mucosa may be modified. This is in part a function of resistance to hydrolysis,

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because penicillin which is broken down by gastric acid will, of course, not be available to be absorbed farther down the intestinal tract. But there may also be a modification of the passage of the intact penicillin across the gastrointestinal mucosa. For example, cloxacillin is much better absorbed than is oxacillin, although the absorption is also more variable.[5] It has been suggested that this may reflect a difference in the ease with which the different penicillins are picked up by some active transport system in the intestinal mucosa and transferred to the plasma. This point is currently under investigation. It is not really known with any certainty that there is in fact an active transport system for penicillins, but it is a topic that is now being explored.

Protein binding is another feature which may be modified by the semisynthetic alteration of the side-chain. This is very important because it may dramatically affect the antibacterial activity of the penicillin. For example, early in the history of the penicillins it was learned that penicillin K, which had a side chain (R) consisting of a simple heptyl group (a straight chain of seven carbon atoms), was very active *in vitro* against bacteria, but was virtually inactive *in vivo*. This was because the straight chain conferred such a strong protein-binding property on the molecule that it remained tightly bound to the serum albumin and was therefore not released to the tissues in which it should have acted. With the semisynthetic penicillins great variations in protein binding are seen. For example, oxacillin is about 88% protein-bound at normal serum protein concentration; cloxacillin is about 94% protein-bound; methicillin, in contrast, is less than 20% bound. The significance of this is that the results of antibiotic activity tests *in vitro*, in the absence of serum, may have little or no relevance to *in vivo* use. Thus, methicillin at any given concentration is almost as active in serum as in an ordinary synthetic medium. But with oxacillin and cloxacillin the concentration must be four to eight times as high in serum as in protein-free media to achieve the same degree of antibacterial activity.[6] Simple measurement of the serum level of penicillin, therefore, may be quite misleading in terms of the anticipated antibacterial effect unless protein binding is taken into account.

Similar considerations apply to the recent synthetic modifications in the structure of cephalosporins. The original molecule of cephalosporin C had an acetyl group (CH_3COO—) as the R′ in Fig. 2, and at R a straight-chain molecule known as α-aminoadipic acid. When the latter was replaced by a cyclic structure (a thiophenyl-methyl group), giving the product known as *cephalothin*, the antibacterial activity was greatly increased. Unfortunately, this was accompanied by a greater instability, the R′ group tending to split off very easily. When the thiophenylmethyl group was kept at R, but the acetyl group at R′ was replaced by another cyclic structure, a pyridyl group, the resulting product (known as *cephaloridine*, or ceporin) proved to be equally active but much more stable.[7] An added gain was a great reduction in protein binding: the required concentration of cephalothin is raised two- to four-fold by the presence of serum, while that of cephaloridine is unaffected.[6] It seems highly probable that many more improvements will be achieved by further modifications of the cephalosporins.

Generally these changes do not greatly affect the manner in which the body disposes of the antibiotics. Penicillins and cephalosporins are not metabolized but are rapidly excreted in the bile, the urine and the milk. They are actively secreted by the renal tubules, by a process that is blocked by probenecid or other tubular blockers. Tubular secretion and glomerular filtration together account for most of the elimination of penicillins and cephalosporins. Protein binding will modify this to some extent, because substances which are protein-bound filter less readily. A curious twist to this is that in patients with renal failure the highly protein-bound cloxacillin is eliminated much more rapidly than methicillin, which is largely free; apparently this is because the liver takes up the protein-bound form more rapidly than the free form, and eliminates it readily in the bile.

Another property of penicillins and cephalosporins which is modified by synthetic changes is their ability to produce sensitivity or allergic reactions. It was originally thought that allergy to penicillins resulted from sensitization to the penicillanic acid nucleus itself (Fig. 1), so that all penicillins should show cross-sensitivity.[8] In other words, a patient sensitive to one penicillin should be allergic to all other penicillins. Cephalosporins, however, will not produce a reaction in such patients. The cephalosporanic acid nucleus (Fig. 2) differs from the penicillanic acid nucleus. There has been a report, however, that some patients who were sensitive to penicillin G were not sensitive to methicillin.[9] This suggested that possibly part of the allergic reaction, or at least the allergic reaction of these patients, might be due not to the penicillin structure itself but to some degradation product of penicillin. One type of degradation reaction already referred to is the formation of penicilloic acids (Fig. 3). Another reaction which penicillins can undergo in water solutions is the rearrangement of the R side group and of the β-lactam ring to give a penicillanic acid. It has been suggested that both products, and possibly others as well, may act as antigens in man, but recent evidence[10] suggests that the penicilloyl derivative is quantitatively the most important in this respect.

Because of the possibility that these degradation products might be involved in allergic reactions, a synthetic antigen was made condensing the penicilloic acid with polylysine, an artificial

material with some of the properties of proteins. This synthetic antigen was found to give a positive skin test reaction in a higher proportion of penicillin-sensitive patients than the native penicillins did.[11, 12] This may offer a possible explanation for reports of non-allergenicity of the penicillinase-resistant penicillins; if the allergy is to a product of bacterial action on the penicillin, then the penicillinase-resistant penicillin might not give rise to the allergen *in vivo*. It is probable that more than one type of allergen is involved because there is by no means a perfect coincidence between clinical allergic reactions and positive sensitivity tests to the synthetic antigen.[11]

Finally, brief mention should be made of the unusual spectrum of activity of one of the most recently developed semisynthetic penicillins, *viz.* ampicillin. The reaction mechanism indicated at the beginning, the formation of a co-valent bond between the β-lactam ring of the penicillin and some site on the bacteria, has been proved only for those bacteria which are normally considered sensitive to penicillins. *E. coli* is not ordinarily considered sensitive to penicillin; yet in very high concentration ordinary penicillin G can be bactericidal to *E. coli*. This apparently is not brought about by covalent bonding to the bacteria, because the penicillin can be washed off. In the presence of the penicillin the bacteria grow into peculiar distorted forms which are osmotically sensitive; but if the penicillin is washed off, the surviving bacteria can revert to their normal form and begin to grow again.

This suggests that there may be a different mechanism of action involved but it also suggests that the effectiveness of different penicillins on different bacteria may be dependent upon the ease with which they can pass through the respective bacterial outer walls to the site of action. In this connection, it is interesting that one group of penicillin derivatives showed degrees of effectiveness which were proportional to their respective lipid solubilities.[13] Ampicillin, the so-called broad-spectrum semisynthetic penicillin, has quite a good effect against *E. coli* and various other organisms normally considered penicillin-insensitive, yet it does not seem to do anything that cannot be achieved with a very much higher concentration of ordinary penicillin G against the same organisms. Therefore it is suggested that the peculiar feature of ampicillin may be that the modification of the side-chain renders it more able to penetrate the Gram-negative bacterial cell wall and get to the site of action.

The points which have been covered in this brief review refer in part to features of the action of modified penicillins and cephalosporins on bacteria, and to an even greater extent to features of the interaction between the modified antibiotics and the host. There is really no basis as yet for a logical prediction about what lines of development are likeliest in the future, because there is room for further improvement in the properties of penicillins and cephalosporins, as well as of other antibiotics, with respect to both their actions on the bacteria and their interactions with the host. What has been covered here is intended merely as a guide to the theoretical basis underlying the alterations made so far, and as an indication that a great deal more is possible in the future.

REFERENCES

1. To-day's Drugs: *Brit. Med. J.*, **2**: 106, 1964.
2. FLEMING, P. C., GOLDNER, M. AND GLASS, D. G.: *Lancet*, **1**: 1399, 1963.
3. CITRI, N. AND GARBER, N.: *J. Pharm. Pharmacol.*, **14**: 784, 1962.
4. New approach to penicillinase problem hinted: *Antibiotic News*, **1**: 6, No. 13, November 25, 1964.
5. SIDELL, S. *et al.*: *Clin. Pharmacol. Ther*, **5**: 26, 1964.
6. BARBER, M. AND WATERWORTH, P. M.: *Brit. Med J.*, **2**: 344, 1964.
7. MUGGLETON, P. W., O'CALLAGHAN, C. H. AND STEVENS, W. K.: *Ibid.*, **2**: 1234, 1964.
8. STEWART, G. T.: *Lancet*, **1**: 509, 1962.
9. LUTON, E. F.: *J. A. M. A.*, **177**: 152, 1961.
10. LEVINE, B. B. AND PRICE, V. H.: *Immunology*, **7**: 542, 1964.
11. BROWN, B. C., PRICE, E. V. AND MOORE, M. B., JR.: *J. A. M. A.*, **189**: 599, 1964.
12. FINKE, S. R. *et al.*: *Amer. J. Med.*, **38**: 71, 1965.
13. HANSCH, C. AND STEWARD, A. R.: *J. Med. Chem.*, **7**: 691, 1964.

SUPPLEMENTARY REFERENCES

For references to topics not covered by the specific papers above, the reader may consult the following excellent reviews:

1. ABRAHAM, E. P.: *Pharmacol. Rev.*, **14**: 473, 1962.
2. GALE, E. F.: *Ibid.*, **15**: 481, 1963.
3. BARBER, M. AND CHAIN, E. B.: *Ann. Rev. Pharmacol.*, **4**: 115, 1964.
4. KIRBY, W. M. M. AND BULGER, R. J.: *Ann. Rev. Med.*, **15**: 393, 1964.

PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

SHELL SHOCK

Men exposed in the trenches to heavy shell fire are inevitably exposed to nerve strain and the effect produced varies within wide limitations. Occasionally there is a somewhat sudden and complete breakdown with results which continue for months. At other times a man may come through all this nervous strain and possibly receive serious wounds without any appreciable collapse of this nature. Some men who suffered in a severe degree appear to be complete wrecks. They are usually very emotional and may break down as they are telling their experiences. Tremor is always a characteristic symptom. The heart's action is rapid, the pulse being from 100 to 120 per minute. The knee jerks are markedly exaggerated—insomnia, anorexia and a constant feeling of fatigue are common. The man is wholly unfit for sustained effort of any kind, his memory is frequently defective and he is obviously worried and apprehensive. These men should have every consideration and should be provided with a suitable environment to ensure recovery. A period of months must elapse before a man who has severe symptoms is fit to assume duty again and occasionally years may elapse before recovery. It is therefore difficult to arrive at a conclusion in endeavouring to predict how soon the individual case will be physically fit for duty. We are told by those at the front that the tendency is to send these men into action too soon, the result being a second breakdown under much less strain than the first. Our impression is that many cases of the exaggerated type will never be fit for active service during the present war.—A. Primrose, *Canad. Med. Ass. J.*, 5: 860, 1915.